09/380638

attach ~~#8~~ #6

VERIFICATION OF A TRANSLATION

I, the below named translator, hereby declare that:

My name and post office address are as stated below:

That I am knowledgeable in the English language and in the language in which the below identified application was filed, and that I believe the English translation of the Japanese Patent Application No. 053409/1997 is a true and complete translation of the above-identified Japanese Patent Application as filed.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated this 9th day of January, 2001.

Full name of the translator: Tadahiko KURITA

Signature of the translator: Tadahiko Kurita

Post Office Address: c/o YUASA AND HARA, Section 206,
New Ohtemachi Bldg., 2-1, Ohtemachi
2-chome, Chiyoda-ku, Tokyo, JAPAN

PATENT OFFICE
JAPANESE GOVERNMENT

This is to certify that the annexed is a true copy of the following application as filed with this Office.

Date of Application: March 7, 1997

Application Number : Patent Application
No. 053409/1997

Applicant(s): Takeshi IMANISHI

Dated this 17th day of April, 1998

Commissioner, Patent Office Hisamitsu ARAI (Seal)

ISSUANCE No. 10-3029439

[Name of Document]	Patent Application
[Case No]	970097
[Filing Date]	March 7, 1997
[To]	Commissioner, Patent Office Mr. Hisamitsu ARAI

[International Patent Classification]

C07H

[Title of the Invention]	NOVEL BICYCLONUCLEOSIDE AND OLIGONUCLEOTIDE ANALOGUE
[Number of the Claims]	5

[Inventor]

[Address]	2-18, Chiyogaoka 2-chome, Nara-shi, Nara
[Name]	Takeshi IMANISHI

[Inventor]

[Address]	2034, Yonbancho, Hiyoshidai, Takatsuki-shi, Osaka
[Name]	Satoshi OBIKA

[Applicant for Patent]

[Identification No.]	594147039
[Name]	Takeshi IMANISHI

[Proxy]

[Identification No.]	100027707
[Patent Attorney]	
[Name]	Kyozo YUASA

[Proxy]

[Identification No.]	100089705
[Patent Attorney]	
[Name]	Ichio SHAMOTO

[Proxy]

[Identification No.]	100071124
[Patent Attorney]	

[Name]	Shosuke IMAI
[Proxy]	
[Identification No.]	100076691
[Patent Attorney]	
[Name]	Chuji MASUI
[Proxy]	
[Identification No.]	100075236
[Patent Attorney]	
[Name]	Tadahiko KURITA
[Proxy]	
[Identification No.]	100075270
[Patent Attorney]	
[Name]	Yasushi KOBAYASHI
[Fees]	
[Page No. of Books]	013228
[Amount]	¥21,000.-

[List of the Documents]

[Item]	Specification	1
[Item]	Drawings	1
[Item]	Abstract	1

[Name of Document] Specification

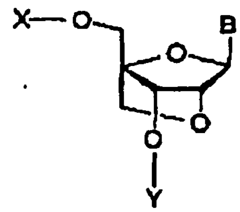
[Title of the Invention]

NOVEL BICYCLONUCLEOSIDE AND OLIGONUCLEOTIDE ANALOGUE

[Claims]

[Claim 1] A nucleoside analogue of the following general formula (I)

[Formula 1]



(I)

where B is a pyrimidine or purine nucleic acid base, or an analogue thereof, and X and Y are identical or different, and each represent a hydrogen atom, an alkyl group, an alkenyl group, an alkinyl group, a cycloalkyl group, an aryl group, an acyl group, or a silyl group,

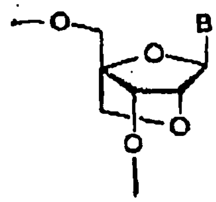
or an amidite derivative thereof.

[Claim 2] A nucleoside analogue as claimed in claim 1, wherein X and Y each represent a hydrogen atom.

[Claim 3] A mononucleoside amidite derivative as claimed in claim 1, wherein X is 4,4-dimethoxytrityl (DMTr), and Y is a 2-cyanoethoxy(diisopropylamino)phosphino group (amidite group).

[Claim 4] An oligonucleotide or polynucleotide analogue having one or more structures of the general formula (Ia)

[Formula 2]

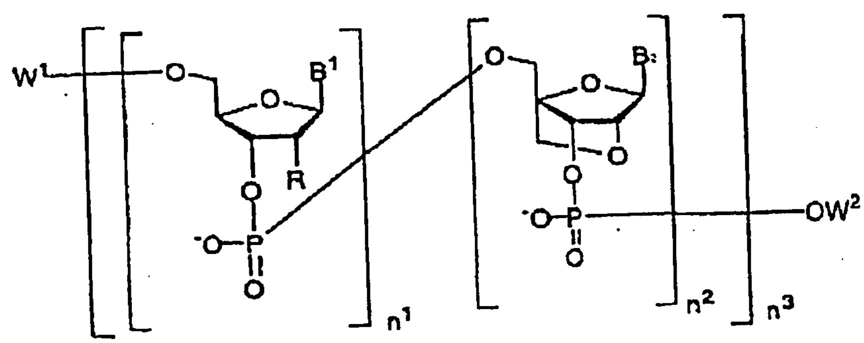


(Ia)

where B is a pyrimidine or purine nucleic acid base, or an analogue thereof.

[Claim 5] An oligonucleotide or polynucleotide analogue of the general formula (II)

[Formula 3]



(II)

where B^1 and B are identical or different, and each represent a pyrimidine or purine nucleic acid base, or an analogue thereof, R is a hydrogen atom, a hydroxyl group, a halogen atom, or an alkoxy group, W^1 and W^2 are identical or different, and each represent a hydrogen atom, an alkyl group, an alkenyl group, an alkinyl group, a cycloalkyl group, an aryl group, an acyl group, a silyl group, a phosphoric acid residue, a naturally occurring nucleoside or

a synthetic nucleoside bound via a phosphodiester bond, or an oligonucleotide or polynucleotide containing the nucleoside, n^1's or n^2's are identical or different, and each denote an integer of 0 to 50, provided that n^1's or n^2's are not zero at the same time, and that not all of n^2's are zero at the same time, n^3 denotes an integer of 1 to 50, provided that when n^1 and/or n^2 are or is 2 or more, B^1 and B need not be identical, and R's need not be identical.

[Detailed Description of the Invention]

[0001]

[Technical Field of the Invention]

This invention relates to a novel nucleoside analogue and a novel nucleotide analogue, and more particularly, to a nucleotide analogue suitable as an antisense molecule.

[0002]

[Prior Art]

In 1978, it was reported for the first time that an antisense molecule inhibited influenza virus infection. Since then, reports have been issued that antisense molecules inhibited the expression of oncogenes and AIDS infection. In recent years, antisense oligonucleotides have become one of the most promising pharmaceuticals, because they specifically control the expression of undesirable genes.

[0003]

The antisense method is based on the idea of controlling a unidirectional flow called the central dogma, i.e., DNA → RNA → protein, by use of an antisense

oligonucleotide.

[0004]

When a naturally occurring oligonucleotide was applied to this method as an antisense molecule, however, it was decomposed with various nucleases in vivo, or its permeation through the cell membrane was not high. To solve these problems, numerous nucleic acid derivatives and analogues have been synthesized, and their studies have been conducted. Examples of the synthesized products include a phosphorothioate having a sulfur atom substituting for an oxygen atom on the phosphorus atom, and a methylphosphonate having a substituting methyl group. Recently, products have been synthesized in which the phosphorus atom has also been substituted by a carbon atom, or the structure of the sugar portion has been changed, or the nucleic acid base has been modified. Any resulting derivatives or analogues, however, have not been fully satisfactory in terms of in vivo stability, ease of synthesis, and sequence specificity (the property of selectively controlling the expression of a particular gene alone).

[0005]

[Problem to be Solved by the Invention]

Under these circumstances, there has been a demand for the creation of an antisense molecule which is minimally decomposed with a nuclease in vivo, binds to target messenger RNA with high affinity, has high specificity, and can thus efficiently control the expression of a particular gene.

[0006]

[Means for Solving the Problems]

The inventors of the present invention designed a nucleic acid analogue with immobilized conformation of the sugar portion in a nucleic acid, which would be useful in the antisense method. They synthesized a nucleoside analogue which will be a unit structure therefor, and confirmed that an oligonucleotide analogue prepared using it was very useful as an antisense molecule.

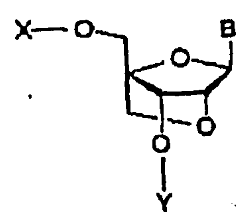
Details of the present invention will now be described.

[0007]

The structure of a nucleoside analogue according to the present invention is a nucleoside analogue of the following general formula (I)

[0008]

[Formula 4]



(I)

where B is a pyrimidine or purine nucleic acid base, or an analogue thereof, and X and Y are identical or different, and each represent a hydrogen atom, an alkyl group, an alkenyl group, an alkinyl group, a cycloalkyl group, an aryl group, an acyl group, or a

silyl group,

or an amidite derivative thereof.

[0009]

The alkyl group represents a straight chain or branched chain alkyl group with 1 to 20 carbon atoms. Its examples include methyl, ethyl, n-propyl, i-propyl, n-butyl, t-butyl, pentyl, hexyl, heptyl, octyl, nonyl and decyl.

[0010]

The alkenyl group represents a straight chain or branched chain alkenyl group with 2 to 20 carbon atoms. Its examples include vinyl, allyl, butenyl, pentenyl, geranyl, and farnesyl.

[0011]

The alkinyl group represents a straight chain or branched chain alkinyl group with 2 to 20 carbon atoms. Its examples include ethynyl, propynyl, and butynyl.

[0012]

The cycloalkyl group represents a cycloalkyl group with 3 to 8 carbon atoms, and includes, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl. Another example is a heterocyclic group in which one or more arbitrary methylene groups on the ring of the cycloalkyl group have been substituted by an oxygen atom, a sulfur atom, or an alkyl-substituted nitrogen atom. It is, for instance, a tetrahydropyranyl group.

[0013]

The aryl group refers to a monovalent substituent formed by removing one hydrogen atom from an aromatic

hydrocarbon group. It includes, for example, phenyl, tolyl, xylyl, biphenyl, naphthyl, anthryl, and phenanthryl. The carbon atom on the ring of the aryl group may be substituted by one or more of a halogen atom, a lower alkyl group, a hydroxyl group, an alkoxyl group, an amino group, a nitro group, and a trifluoromethyl group. The substituent in this case is, for example, a halogen atom, a hydroxyl group, an amino group, an alkoxy group, or an aryloxy group.

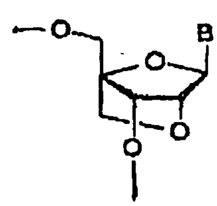
[0014]

As the acyl group, acetyl, formyl, propionyl, benzoyl, and benzyloxycarbonyl can be exemplified. An example of the silyl group is a trialkylsilyl group, preferably trimethylsilyl, triethylsilyl, triisopropylsilyl, t-butyldimethylsilyl or t-butyldiphenylsilyl, and more preferably trimethylsilyl.

[0015]

The nucleotide analogue of the present invention is an oligonucleotide or polynucleotide analogue having one or more structures of the general formula (Ia)

[Formula 5]



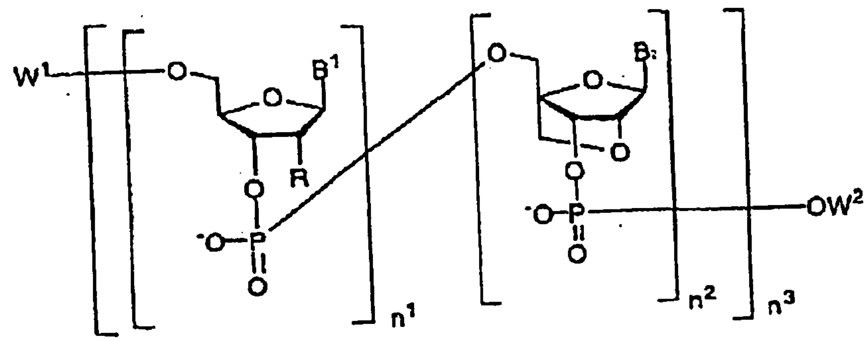
(Ia)

where B is a pyrimidine or purine nucleic acid base,

or an analogue thereof,

or an oligonucleotide or polynucleotide analogue of the general formula (II)

[Formula 6]



(II)

where B^1 and B are identical or different, and each represent a pyrimidine or purine nucleic acid base, or an analogue thereof, R is a hydrogen atom, a hydroxyl group, a halogen atom, or an alkoxy group, W^1 and W^2 are identical or different, and each represent a hydrogen atom, an alkyl group, an alkenyl group, an alkinyl group, a cycloalkyl group, an aryl group, an acyl group, a silyl group, a phosphoric acid residue, a naturally occurring nucleoside or a synthetic nucleoside bound via a phosphodiester bond, or an oligonucleotide or polynucleotide containing the nucleoside, n^1's or n^2's are identical or different, and each denote an integer of 0 to 50, provided that n^1's or n^2's are not zero at the same time, and that not all of n^2's are zero at the same time, n^3 denotes an integer of 1 to 50, provided that

when n^1 and/or n^2 are or is 2 or more, B^1 and B need not be identical, and R's need not be identical.

[0016]

The pyrimidine or purine nucleic acid base in the present invention refers to thymin, uracil, cytosine, adenine, guanine, or a derivative thereof.

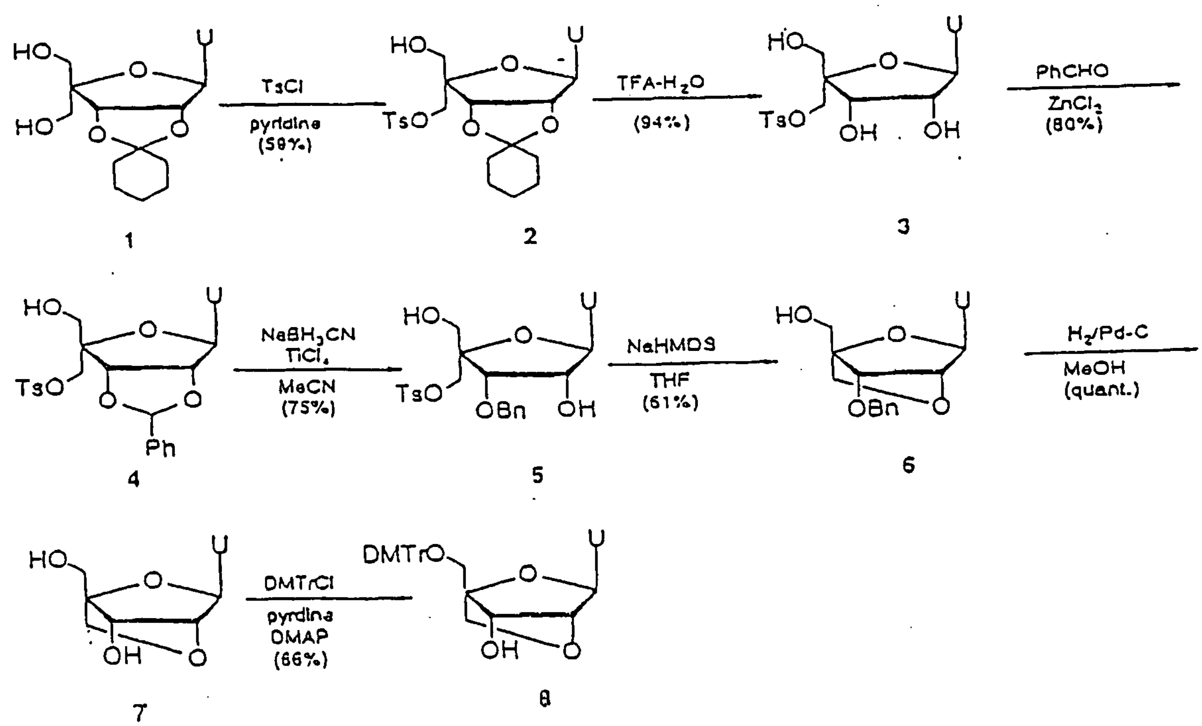
[0017]

The nucleoside analogue and nucleotide analogue of the present invention can be synthesized in the manner described below.

[0018]

(1) Synthesis of nucleoside analogue

[Formula 7]



Compound 1, synthesized from uridine in accordance with the literature [1) J.A. Secrist et al., J. Am. Chem.

Soc., 101, 1554 (1979); 2) G.H. Jones et al., J. Org. Chem., 44, 1309 (1979)], was treated with tosyl chloride (TsCl) to tosylate only one of the two primary alcohols, leading to Compound 2. Compound 2 was acid hydrolyzed into a triol compound 3. Compound 3 was condensed with benzaldehyde in the presence of an acid catalyst to form a benzylidene compound 4. Compound 4 was reduced with sodium cyanoborohydride ($NaBH_3CN$) in the presence of titanium tetrachloride ($TiCl_4$) to obtain Compound 5. This compound was reacted with sodium hexamethyldisilazide (NaHMDS) in tetrahydrofuran (THF) to obtain a bicyclo compound 6 (Compound I: B = uracil (U), X = H, Y = benzyl). When Compound 6 was catalytically reduced in the presence of a palladium carbon catalyst, a diol compound 7 (Compound (I): B = U, X = Y = H) was obtained. Further treatment of Compound 7 with 4,4'-dimethoxytrityl chloride (DMTrCl) gave a trityl compound 8 (Compound I: B = U, X = DMTr, Y = H). Compounds 6, 7 and 8 can be used as starting materials for various compounds I.

[0019]

Compounds (I) having various nucleic acid bases, whether natural or nonnatural, other than uridine, can be synthesized by any of the following two methods:

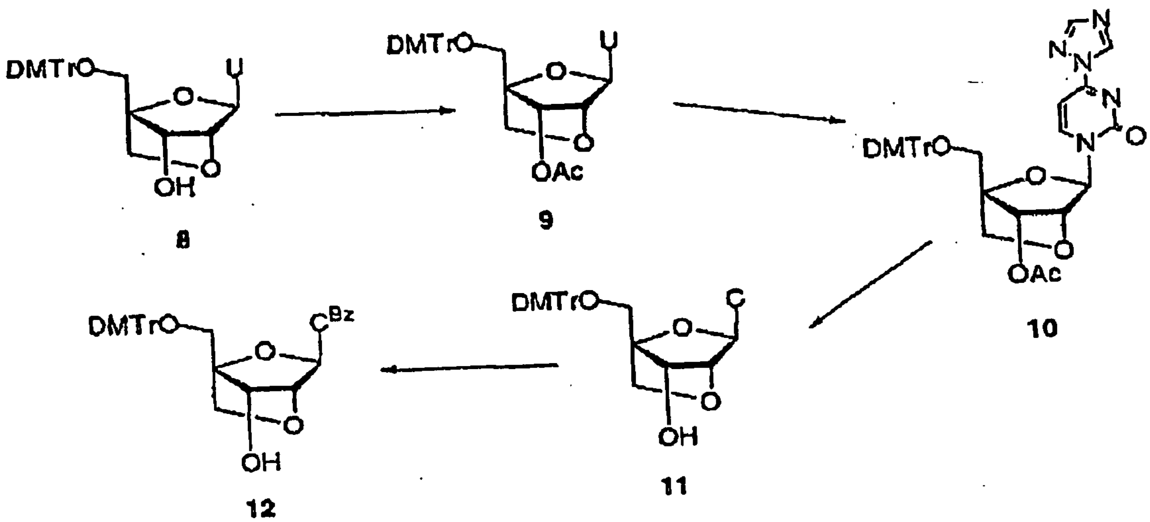
[0020]

The first method is conversion from Compound 8. That is, Compound 8 is acetylated into Compound 9, and then reacted with 1,2,4-triazole to form Compound 10. Hydrolysis of this compound gave Compound 11 (Compound (I): B = cytosine (C), X = DMTr, Y = H). Compound 12 (Compound (I):

B = benzoylcytosine (C^{Bz}), X = DMTr, Y = H), which will become a starting material for oligonucleotide synthesis, can be easily obtained by benzoylation of Compound 11.

[0021]

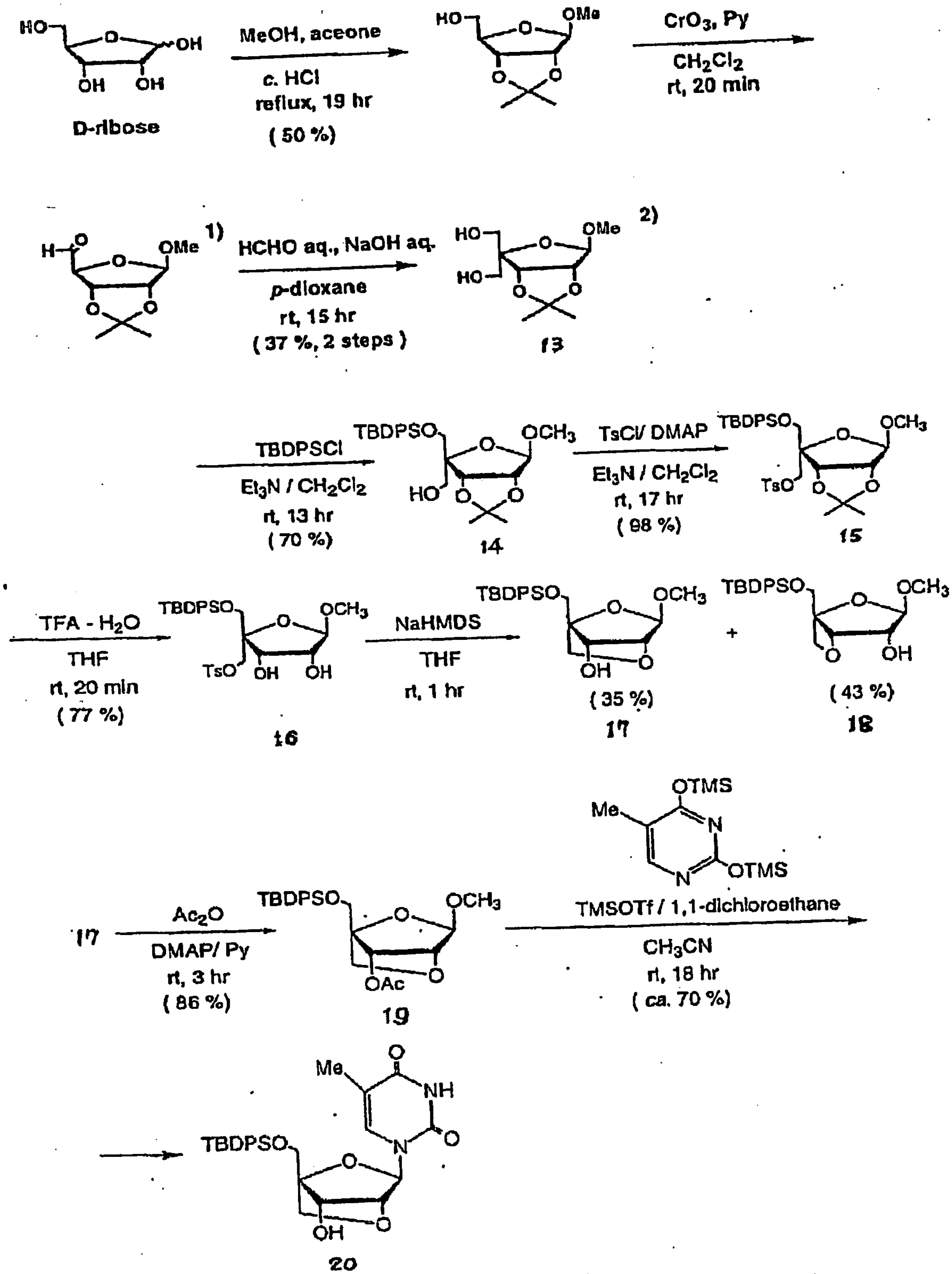
[Formula 8]



The second method is a method performed via Compound 13 which can be easily obtained from D-ribose in accordance with the literature [3) A.G.M. Barrett et al., J. Org. Chem., 55, 3853 (1990); 4) G.H. Jones et al., ibid., 44, 1309 (1979)]. That is, Compound 13 was led to Compound 16 by three steps, and cyclized under basic conditions to obtain a desired methylglycosyl compound 17. The OMe group at the 1-position of this compound can be substituted by different natural nucleic acid bases or nonnatural nucleic acid base analogues by various methods which have already been developed. For example, a method as shown by a scheme ranging from Compound 17 to Compound 20 can be employed.

[0022]

[Formula 9]

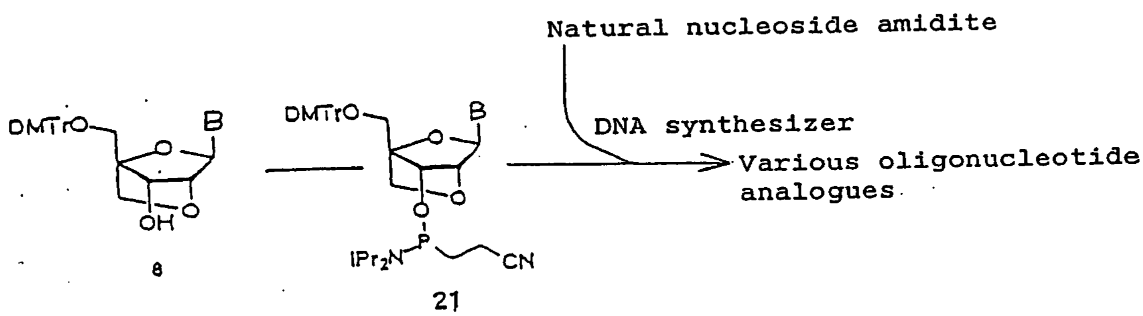


(2) Synthesis of oligonucleotide analogue

Compound 8 is reacted with 2-cyanoethyl-N,N,N',N'-tetraisopropylphosphoramidite to obtain an amidite compound 16. This compound is combined with a naturally occurring nucleoside amidite, and subjected to a DNA synthesizer to synthesize various oligonucleotide analogues. The synthesized crude products are purified using a reversed phase chromatographic column (Oligo-Pak). The purity of the purified product is analyzed by HPLC, whereby the formation of a purified oligonucleotide analogue can be confirmed.

[0023]

[Formula 10]



At least one monomer unit as compound 8 can be contained in the oligonucleotide analogue. Alternatively, the monomer units may be present at two or more locations in the oligonucleotide analogue in such a manner as to be separated from each other via one or more naturally occurring nucleotides. The present invention makes it possible to synthesize an antisense molecule incorporating a necessary number of the nucleotide analogues (nucleoside analogues) of the invention (a necessary length of the nucleotide or nucleoside analogue) at a necessary location.

The length of the entire oligonucleotide analogue is 2 to 50, preferably 10 to 30, nucleoside units.

[0024]

Such an oligonucleotide analogue (antisense molecule) is minimally degradable by various nucleases, and can be existent in vivo for a long time after administration. This antisense molecule functions, for example, to form a stable double helix together with a messenger RNA, thereby inhibiting the biosynthesis of a potentially pathogenic protein; or form a triple helix in combination with double-stranded DNA in a genome to inhibit transcription to messenger RNA. The oligonucleotide analogue can also suppress the proliferation of a virus which has infected.

[0025]

In light of these findings, an oligonucleotide analogue (antisense molecule) using the nucleoside analogue of the present invention is expected to be useful as drugs, including antineoplastics and antivirals, for treatment of diseases by inhibiting the actions of particular genes.

[0026]

The antisense molecule using the nucleotide (nucleoside) analogue of the present invention can be formulated into parenteral preparations or liposome preparations by incorporating customary auxiliaries such as buffers and/or stabilizers. As preparations for topical application, it may be blended with pharmaceutical carriers in common use to prepare ointments, creams, liquids or plasters.

[0027]

[Examples]

Synthesis of the nucleoside analogue and nucleotide analogue of the present invention will be described in more detail by way of the following Examples and Production Examples.

[0028]

Example 1: Synthesis of nucleoside analogue

(1) Synthesis of 2',3'-O-cyclohexylidene-4'-(p-toluenesulfonyloxymethyl)uridine (Compound 2)

To an anhydrous pyridine solution (13.5 ml) of Compound 1 (956 mg, 2.70 mmols) known in the literature, p-toluenesulfonyl chloride (771 mg, 4.05 mmols) was added at room temperature in a stream of nitrogen, and the mixture was stirred for 5 hours at 60°C.

[0029]

To the reaction mixture, a saturated sodium bicarbonate solution was added, whereafter the reaction system was extracted with benzene 3 times. The organic phase was washed once with a saturated sodium chloride solution, and dried over anhydrous $MgSO_4$. The solvents were distilled off under reduced pressure, and the residue was subjected to azeotropy with benzene 3 times. The resulting crude product was purified by silica gel column chromatography ($CHCl_3$:MeOH = 15:1), and then reprecipitated from benzene-hexane to obtain a white powder (Compound 2) (808 mg, 1.59 mmols, 59%).

[0030]

Compound 2: White powder, m.p. 104-106°C (benzene-hexane). IR ν (KBr): 3326, 2929, 2850, 1628, 1577, 1544, 1437, 1311, 1244 cm^{-1}. ^1H-NMR (d_6-acetone): δ 1.45-1.67 (10H, m), 2.45 (3H, s), 3.71 (2H, ABq, J = 12 Hz), 4.20 (2H, ABq, J = 11 Hz), 4.92 (1H, d, J' = 6 Hz), 5.05, 5.06 (1H, dd, J = 4.6 Hz), 5.60 (1H, d, J = 7 Hz), 5.75 (1H, d, J = 4 Hz), 7.48 (2H, d, J = 8 Hz), 7.77 (1H, d, J = 8 Hz), 7.81 (2H, d, J = 8 Hz), 10.10 (1H, s,). ^{13}C-NMR (d_6-acetone): δ 21.5, 24.1, 24.5, 25.5, 34.8, 36.9, 63.5, 69.7, 82.5, 84.7, 87.8, 92.9, 102.9, 115.4, 128.8, 130.8, 133.9, 142.7, 145.9, 151.3, 163.5. Mass (EI): m/z 481 (M^+-H_2O).

Anal. Calcd. for $C_{23}H_{28}N_2O_9S \cdot 1/3\ H_2O$: C, 53.69; H, 5.61; N, 5.44; S, 6.22. Found: C, 53.99;H, 5.48;N, 5.42;S, 6.10.

(2) Synthesis of 4'-(p-toluenesulfonyloxymethyl)uridine (Compound 3)

The above compound 2 (107 mg, 0.21 mmol) was stirred in TFA-H_2O (98:2, 1 ml) for 10 minutes at room temperature. The reaction mixture was distilled off under reduced pressure, and EtOH was added to the residue, followed by performing azeotropy 3 times. The resulting crude product was purified by silica gel column chromatography ($CHCl_3$:MeOH = 10:1) to obtain Compound 3 (85.0 mg, 0.20 mmol, 94%).

[0031]

Compound 3: White powder, m.p. 119-120°C. IR ν (KBr): 3227, 3060, 2932, 2837, 1709, 1508, 1464, 1252, 978, 835, 763, 556 cm^{-1}. ^1H-NMR (d_6-acetone): δ 2.31 (3H, s), 2.84 (3H, s), 3.71 (2H, s), 4.13, 4.20 (2H, ABq, J = 11 Hz), 4.28, 4.31 (1H, dd, J' = 9.6 Hz), 4.36 (1H, d, J' = 6 Hz), 5.54

(1H, d, J' = 8 Hz), 5.75 (1H, d, J = 7 Hz), 7.32 (2H, d, J = 8 Hz), 7.67 (2H, d, J = 8 Hz), 7.70 (1H, d, J' = 8 Hz), 10.14 (1H, s). ^{13}C-NMR (d_6-acetone): δ 21.5, 63.7, 70.8, 72.7, 74.6, 86.8, 88.8, 103.1, 128.8, 130.7, 133.9, 141.7, 145.8, 151.8, 163.9. Mass (EI): m/z 256 (M^+-OTs).

(3) Synthesis of 2',3'-O-benzylidene-4'-(p-toluenesulfonyloxymethyl)uridine (Compound 4)

In a stream of nitrogen, benzaldehyde (2.4 ml, excess) and zinc chloride (670 mg, 5.0 mmols) were added to the above compound 3 (400 mg, 0.93 mmols), and the mixture was stirred for 5 hours at room temperature. After the reaction was stopped by addition of a saturated sodium bicarbonate solution, the reaction mixture was extracted with chloroform, and washed with a saturated sodium bicarbonate solution, water, and a saturated sodium chloride solution. The organic phase was dried over anhydrous sodium sulfate. The solvents were distilled off under reduced pressure, and the residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 40:1) to obtain Compound 4 (380 mg, 0.74 mmol, 80%).

[0032]

Compound 4: White powder. m.p. 99-102°C (CH_2Cl_2-hexane). $[\alpha]_D^{23}$-26.7° (c = 1.0, $CHCl_3$). IR ν (KBr): 3059, 1691, 1460, 1362, 1269, 1218, 1177 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 2.41 (3H, s), 3.25 (1H, br), 3.79 (2H, m), 4.19 (2H, s), 5.09 (1H, d, J = 7 Hz), 5.28 (1H, dd, J = 3.7 Hz), 5.60 (1H, d, J = 4 Hz), 5.73 (1H, d, J = 8 Hz), 5.94 (1H, s), 7.24 (1H, d, J = 8 Hz), 7.38 (2H, d, J = 9 Hz), 7.42 (5H, br), 7.69

(2H, d, J = 9 Hz), 9.11 (1H, br). ^{13}C-NMR ($CDCL_3$): δ 21.6, 63.5, 68.3, 77.2, 82.8, 84.2, 87.7, 94.9, 102.6, 107.5, 126.5, 127.9, 128.5, 129.7, 132.2, 135.0, 143.0, 145.0, 150.4, 163.5.

Anal. Calcd. for $C_{24}H_{24}N_2O_9S \cdot 1/3\ H_2O$: C, 55.17; H, 4.76; N, 5.36; S, 6.14. Found: C, 55.19;H, 4.66;N, 5.29;S, 5.98.

(4) Synthesis of 3'-O-benzyl-4'-(p-toluenesulfonyloxymethyl)uridine (Compound 5)

To an acetonitrile solution (3 ml) of Compound 4 (150 mg, 0.29 mmol), sodium borocyanohydride (92 mg, 1.5 mmols) was added at room temperature in a stream of nitrogen. Then, titanium tetrachloride (0.16 ml, 1.5 mmols) was added dropwise under cooling with ice, and the mixture was stirred for 15 hours at room temperature. The reaction mixture was diluted with chloroform, and washed with a saturated sodium bicarbonate solution, water, and a saturated sodium chloride solution. Then, the organic phase was dried over anhydrous sodium sulfate. After the solvents were distilled off, the residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 25:1) to obtain Compound 5 (112 mg, 0.22 mmol, 75%).

[0033]

Compound 5: Colorless crystals. m.p. 195-197°C (AcOEt-hexane). $[\alpha]_D^{23}$ -14.6° (c = 1.0, $CHCl_3$). IR ν (KBr): 3033, 2885, 2820, 1726, 1470, 1361, 1274, 1175, 1119 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.40 (3H, s), 3.59-3.77 (3H, m), 4.10, 4.24 (2H, AB, J = 11 Hz), 4.32 (1H, d, J = 6 Hz), 4.56 (2H, m), 4.69 (1H, d, J = 11 Hz), 5.52 (1H, d, J = 6 Hz), 5.67

(1H, d, J = 8 Hz), 7.24-7.29 (7H, m), 7.48 (1H, d, J = 8 Hz), 7.70 (2H, d, J = 9 Hz), 9.91 (1H, s). ^{13}C-NMR ($CDCl_3$): δ 21.6, 63.2, 69.2, 73.6, 74.6, 78.1, 86.6, 92.9, 102.5, 127.9, 128.2, 128.3, 128.6, 129.9, 132.3, 136.9, 142.4, 145.2, 150.7, 163.8.

Anal. Calcd. for $C_{24}H_{26}N_2O_9S$: C, 55.59; H, 5.05; N, 5.40; S, 6.18. Found: C, 55.41;H, 5.02;N, 5.32;S, 6.15.

(5) Synthesis of 3'-O-benzyl-2'-O, 4'-methyleneuridine (Compound 6)

To an anhydrous THF solution (1.5 ml) of Compound 5 (80 mg, 0.16 mmol), an anhydrous benzene suspension (0.7 ml) of NaHMDS (3.2 mmols) was added at room temperature in a stream of nitrogen, and the mixture was stirred for 20 hours at room temperature. A saturated sodium bicarbonate solution was added to the reaction mixture, followed by extracting the mixture with $CHCl_3$. The organic phase was washed with a saturated sodium chloride solution, and then dried over anhydrous sodium sulfate. After the solvents were distilled off under reduced pressure, the resulting crude product was purified by silica gel column chromatography ($CHCl_3$:MeOH = 10:1), and then recrystallized from MeOH to obtain Compound 6 (41 mg, 0.10 mmol, 61%).

[0034]

Compound 6: Colorless crystals. m.p. 217-219°C (MeOH). $[\alpha]_D^{23}$+108.4° (c = 0.3, MeOH). IR ν (KBr): 3059, 2951, 1688, 1459, 1271, 1053 cm^{-1}. ^{1}H-NMR (d_6-DMSO) δ: 3.75, 3.85 (2H, AB, J = 8 Hz), 3.77 (2H, d, J = 5 Hz), 3.92 (1H, s), 4.44 (1H, s), 4.60 (2H, s), 5.39 (1H, t, J = 5 Hz), 5.48

(1H, s), 7.31 (5H, m), 7.72 (1H, d, J = 8 Hz), 11.37 (1H, s). ^{13}C-NMR (d_6-DMSO) δ: 56.0, 71.1, 71.6, 75.8, 76.5, 86.5, 88.3, 100.9, 127.4, 127.6, 128.2, 137.9, 139.0, 150.0, 163.3.
Mass (EI): m/z 346 (M^+, 1.1).

Anal. Calcd. for $C_{17}H_{18}N_2O_6$: C, 58.96; H, 5.24; N, 8.09. Found: C, 58.67;H, 5.23;N, 8.05.

(6) Synthesis of 2'-O,4'-methyleneuridine (Compound 7)

To a methanol solution (2.5 ml) of Compound 6 (25 mg, 0.072 mmol), 10% Pd-C (25 mg) was added, and the mixture was stirred for 15 hours at atmospheric pressure in a stream of hydrogen. The reaction mixture was filtered, and the solvent was distilled off. Then, the residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 10:1, then 5:1) to obtain Compound 7 (18.3 mg, quant.).

[0035]

Compound 7: Colorless crystals. m.p. 239-243°C (MeOH). $[\alpha]_D^{23}$+92.2° (c = 0.3, MeOH). IR ν (KBr): 3331, 3091, 3059, 2961, 1689, 1463, 1272, 1049 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 3.76, 3.96 (2H, AB, J = 8 Hz), 3.90 (2H, s), 4.04 (1H, s), 4.28 (1H, s), 5.55 (1H, s), 5.69 (1H, d, J = 8 Hz), 7.88 (1H, d, J = 8 Hz).

Anal. Calcd. for $C_{10}H_{12}N_2O_6$: C, 46.88; H, 4.72; N, 10.93. Found: C, 46.74;H, 4.70;N, 10.84.

(7) 5'-O-(4,4'-dimethoxytrityl)-2'-O,4'-methyleneuridine (Compound 8)

To Compound 7 (140 mg, 0.53 mmol), anhydrous pyridine was added, followed by performing azeotropy of the mixture 3 times. Then, the product was converted into an anhydrous

pyridine solution (1.5 ml), and 4,4'-dimethoxytrityl chloride (210 mg, 0.63 mmol) and DMAP (6.5 mg, 0.053 mmol) were added at room temperature in a stream of nitrogen. The mixture was stirred for 5 hours at room temperature. To the reaction mixture, a saturated sodium bicarbonate solution was added, followed by extraction with CH_2Cl_2. The organic phase was washed with water and a saturated sodium chloride solution, and then dried over anhydrous sodium sulfate. After the solvents were distilled off under reduced pressure, the resulting crude product was purified by silica gel column chromatography ($CHCl_3$:MeOH = 40:1) to obtain Compound 8 (230 mg, 0.34 mmol, 66%).

[0036]

Compound 8: White powder. m.p. 117-120°C ($CHCl_3$). $[\alpha]_D^{23}$+17.2° (c = 1.0, $CHCl_3$). IR ν (KBr): 3393, 3101, 2885, 1689, 1464, 1272, 1047 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.59 (1H, br), 3.56 (2H, q, J = 7, 11 Hz), 3.87 (1H, d, J = 7 Hz), 4.26 (1H, s), 4.47 (1H, s), 5.60 (1H, d, J = 9 Hz), 5.63 (1H, s), 5.84 (4H, d, J = 9 Hz), 7.22-7.45 (9H, m), 7.93 (1H, d, J = 9 Hz).

Example 2: Synthesis of nucleoside analogue

(1) Synthesis of methyl=5'-O-(t-butyldiphenylsilyl)-4'-hydroxymethyl-2',3'-O-isopropylidene-β-D-ribofuranoside (Compound 14)

In a stream of nitrogen, Et_3N (2.62 ml, 18.8 mmols) and t-butyldiphenylsilyl chloride (4.88 ml, 18.8 mmols) were added to an anhydrous CH_2Cl_2 solution (40 ml) of Compound 13 (2.00 g, 8.54 mmols) known in the literature under cooling with ice, and the mixture was stirred for 13 hours at room

temperature. To the reaction mixture, a saturated sodium bicarbonate solution was added, whereafter the reaction system was extracted with AcOEt 3 times. The organic phase was washed once with a saturated sodium chloride solution, and then dried over anhydrous Na_2SO_4. The solvents were distilled off under reduced pressure, and the resulting crude product was purified by silica gel column chromatography (hexane:AcOEt = 5:1) to obtain colorless oily matter (Compound 14) (2.82 g, 5.98 mmols, 70%).

[0037]

$[\alpha]_D^{17}$-16.2° (c = 0.52, $CHCl_3$). IR ν (KBr): 3510, 3061, 2938, 2852, 1465, 1103 cm^{-1}.

^{1}H-NMR ($CDCl_3$) δ: 1.09 (9H, s), 1.28 (3H, s), 1.49 (3H, s), 3.22 (3H, s), 3.67, 3.76 (2H, AB, J = 11 Hz), 3.88, 3.93 (2H, AB, J = 11 Hz), 4.49 (1H, d, J = 6 Hz), 4.57 (1H, d, J = 6 Hz), 4.93 (1H, s), 7.38 - 7.43 (6H, m), 7.67 (4H, d, J = 7 Hz).

^{13}C-NMR ($CDCl_3$) δ_c: 19.2, 24.4, 25.9, 26.9, 55.0, 62.9, 64.8, 82.2, 85.9, 88.7, 108.6, 112.6, 127.8, 129.9, 133.0, 135.7.

Anal. Calcd. for $C_{26}H_{36}O_6Si\cdot 1/4\ H_2O$: C, 65.45; H, 7.71. Found: C, 65.43; H, 7.59.

(2) Synthesis of methyl=5'-O-(t-butyldiphenylsilyl)-2',3'-O-isopropylidene-4'-(p-toluenesulfonyloxymethyl)-β-ribofuranoside (Compound 15)

In a stream of nitrogen, Et_3N (3.92 g, 28.0 mmols), p-toluenesulfonyl chloride (1.34 g, 7.22 mmols), and 4-dimethylaminopyridine (90 mg, 0.72 mmol) were added to an anhydrous CH_2Cl_2 solution (15 ml) of Compound 14 (2.13 g,

4.51 mmols), and the mixture was stirred for 17 hours at room temperature. To the reaction mixture, a saturated sodium bicarbonate solution was added, whereafter the reaction system was extracted with AcOEt 3 times. The organic phase was washed once with a saturated sodium chloride solution, and then dried over anhydrous Na_2SO_4. The solvents were distilled off under reduced pressure, and the resulting crude product was purified by silica gel column chromatography (hexane:AcOEt = 10:1) to obtain colorless oily matter, Compound 15 (2.76 g, 4.42 mmols, 98%).

[0038]

$[\alpha]_D^{17}$-3.82° (c = 0.56, $CHCl_3$). IR ν (KBr): 2934, 2852, 1369, 1104 cm^{-1}.

^{1}H-NMR ($CDCl_3$) δ: 1.02 (9H, s), 1.20 (3H, s), 1.32 (3H, s), 2.41 (3H, s), 3.09 (3H, s), 3.51, 3.77 (2H, AB, J = 10 Hz), 4.34 (1H, d, J = 6 Hz), 4.25, 4.39 (2H, AB, J = 9 Hz), 4.47 (1H, d, J = 6 Hz), 4.77 (1H, s), 7.28, 7.81 (4H, AB, J = 9 Hz), 7.39 - 7.44 (6H, m), 7.62 - 7.65 (4H, m), 7.81 (2H, d, J = 9 Hz).

^{13}C-NMR ($CDCl_3$) δ_c: 19.2, 21.6, 24.5, 25.8, 26.8, 54.9, 62.7, 68.8, 81.9, 85.6, 87.5, 108.7, 112.8, 127.7, 127.8, 128.2, 129.6, 129.9, 132.9, 135.6, 144.4.

Anal. Calcd. for $C_{33}H_{42}O_8SSi$: C, 63.23; H, 6.75; S, 5.11. Found: C, 62.99; H, 6.53; S, 5.13.

(3) Synthesis of methyl=5'-O-(t-butyldiphenylsilyl)-4'-(p-toluenesulfonyloxymethyl)-β-D-ribofuranoside (Compound 16)

Trifluoroacetic acid (14 ml) was added to a THF-H_2O

[11 ml, 8:3 (v/v)] solution of Compound 15 (645 mg, 1.03 mmols) at room temperature, and the mixture was stirred for 20 minutes at room temperature. The solvents were distilled off under reduced pressure, and the resulting crude product was purified by silica gel column chromatography (hexane:AcOEt = 5:1) to obtain colorless oily matter, Compound 16 (464 mg, 0.79 mmol, 77%).

[0039]

$[\alpha]_D^{17}$-35.8° (c=1.90, $CHCl_3$) IR ν (KBr):3499, 3051, 2931, 2840, 1594, 1468,1362, 1109cm^{-1}.

^{1}H-NMR ($CDCl_3$)δ : 1.02(9H,s), 2.42(3H,s), 3.16(3H,s), 3.54, 3.70(2H,AB,J=10Hz), 3.97(1H,d,J=5Hz), 4.18(1H,d,J=5Hz), 4.26, 4.39(2H,AB,J=10Hz), 4.73(1H,s), 7.30(2H,d,J=8Hz), 7.36-7.44 (6H,m), 7.59-7.66(4H,m),7.78(2H,d,J=8Hz). ^{13}C-NMR ($CDCl_3$) δ_c : 19.2, 21.6, 26.7, 55.2, 66.5, 69.6, 74.0, 75.2, 76.5, 84.8, 107.5, 127.7, 128.0, 129.8, 132.6, 132.7, 132.8, 135.5, 135.6, 144.9.

Anal. Calcd for $C_{30}H_{38}SSiO_8 \cdot 1/4\ H_2O$:C,60.94; H,6.56.Found:C, 60.94; H,6.43.

(4) Synthesis of methyl=5'-O-(t-butyldiphenylsilyl)-2'-O,4'-methylene-β-D-ribofuranoside (Compound 17) and methyl=5'-O-(t-butyldiphenylsilyl)-3'-O,4'-methylene-β-D-ribofuranoside (Compound 18)

In a stream of nitrogen, a benzene suspension (1.6 ml) of NaHMDS (3.30 mmols) was added to an anhydrous THF solution (4 ml) of Compound 16 (194 mg, 0.33 mmol) at room temperature, and the mixture was stirred for 1 hour at room temperature. After a saturated sodium bicarbonate solution

was added to the reaction mixture, the reaction solvents were distilled off, and the residue was extracted with AcOEt 3 times. The organic phase was washed once with a saturated sodium chloride solution, and then dried over anhydrous Na_2SO_4. The solvent was distilled off under reduced pressure, and the resulting crude product was purified by silica gel column chromatography (hexane:AcOEt = 5:1) to obtain colorless oily matter, Compound 17 (48 mg, 0.116 mmol, 35%) and colorless oily matter, Compound 18 (59 mg, 0.142 mmol, 43%).

[0040]

Compound 17: IR ν (KBr):3438, 3064, 1103, 1036cm^{-1}.

^{1}H-NMR ($CDCl_3$)δ: 1.08(9H,s), 2.04(1H,br s), 3.39(3H,s), 3.65, 3.98(2H,AB,J=8Hz), 3.95,4.02(2H,AB,J=12Hz), 4.02(1H,s), 4.30 (1H,s), 4.79(1H,s), 7.38-7.46(6H,m), 7.65-7.69(4H,m).

^{13}C-NMR ($CDCl_3$) δ_c: 19.2, 26.7, 55.0, 60.7, 71.2, 73.1, 79.9, 85.5, 104.3, 127.8, 129.9, 130.0, 132.9, 135.6, 135.7.

Anal.Calcd for $C_{23}H_{30}O_5Si \cdot 1/4\ H_2O$:C,65.68; H,7.34.Found:C,65.98; H,7.23.

Compound 18: IR ν (KBr):3456, 3058, 2938, 2852, 1467, 1108cm^{-1}.

^{1}H-NMR ($CDCl_3$)δ: 1.10(9H,s), 3.26(3H,s), 3.71(2H,s), 4.02(1H,d, J=6Hz), 4.35,4.95(2H,d,J=7Hz), 5.01(1H,s), 5.11(1H,d,J=6Hz), 7.38-7.44(6H,m), 7.66(4H,d,J=7Hz).

^{13}C-NMR($CDCl_3$)δ_c: 19.3, 26.8, 55.4, 63.7, 75.1, 77.9, 84.5, 86.3, 111.9, 127.8, 128.0, 129.9, 132.9, 133.0, 135.6, 135.8, 135.9.

Anal.Calcd for $C_{23}H_{30}O_5Si \cdot 1/4\ H_2O$:C,65.91; H,7.34.Found:C, 66.07; H,7.14.

(5) Synthesis of methyl=3'-O-acetyl-5'-O-(t-butyldiphenylsilyl)-2'-O,4'-methylene-β-D-ribofuranoside (Compound 19)

In a stream of nitrogen, acetic anhydride (0.38 ml, 4.08 mmols) and 4-dimethylaminopyridine (21 mg, 0.170 mmols) were added to an anhydrous pyridine solution (10 ml) of Compound 17 (704 mg, 1.70 mmols) at room temperature, and the mixture was stirred for 3 hours at room temperature. After a saturated sodium bicarbonate solution was added to the reaction mixture, the system was extracted with AcOEt 3 times. The organic phase was washed once with a saturated sodium chloride solution, and then dried over anhydrous Na_2SO_4. The solvents were distilled off under reduced pressure, and the resulting crude product was purified by silica gel column chromatography (hexane:AcOEt = 7:1) to obtain colorless oily matter, Compound 19 (665 mg, 1.46 mmols, 86%).

$[\alpha]_D^{17}$-34.3° (c=0.93,$CHCl_3$) IR ν (KBr):3438, 3064, 2934, 1749, 1468, 1103, 1036cm^{-1}.

^{1}H-NMR ($CDCl_3$)δ: 0.99(9H,s), 1.97(3H,s), 3.34(3H,s), 3.69, 3.86(2H,AB,J=8Hz), 3.86(2H,s), 4.17(1H,s), 4.77(1H,s), 5.06 (1H,s), 7.28-7.39(6H,m), 7.58-7.63(4H,m).

^{13}C-NMR($CDCl_3$)δ_c: 19.3, 20.9, 26.7, 55.0, 60.3, 72.0, 73.6, 78.3, 85.3, 104.4, 127.7, 129.8, 133.0, 135.6, 169.8.

Anal.Calcd for $C_{25}H_{32}O_6Si\cdot 1/4\ H_2O$:C,65.12; H,7.10. Found:C, 65.27;H,7.00.

(6) Synthesis of 5'-O-(t-butyldiphenylsilyl)-2'-O,4'-methylene-5-methyluridine (Compound 20)

In a stream of nitrogen, O,O'-bistrimethylsilylthymine (154 mg, 0.598 mmols) was added to an anhydrous CH_3CN solution (2 ml) of Compound 19 (109.2 g, 0.239 mmol) at room temperature. Then, a 1,1-dichloroethane (0.31 ml) solution of trimethylsilyltrifluoromethane sulfonate (0.82 ml, 8.74 mmols) was added under cooling with ice, and the mixture was stirred for 18 hours at room temperature. The reaction mixture was diluted with CH_2Cl_2, and a saturated sodium bicarbonate solution was added, followed by extracting the system with AcOEt 3 times. The organic phase was washed once with a saturated sodium chloride solution, and then dried over anhydrous Na_2SO_4. The solvents were distilled off under reduced pressure, and the resulting crude product was purified by silica gel column chromatography (hexane:AcOEt = 3:1) to obtain colorless oily matter, Compound 20 (87.7 mg, 0.173 mmol, 70%).

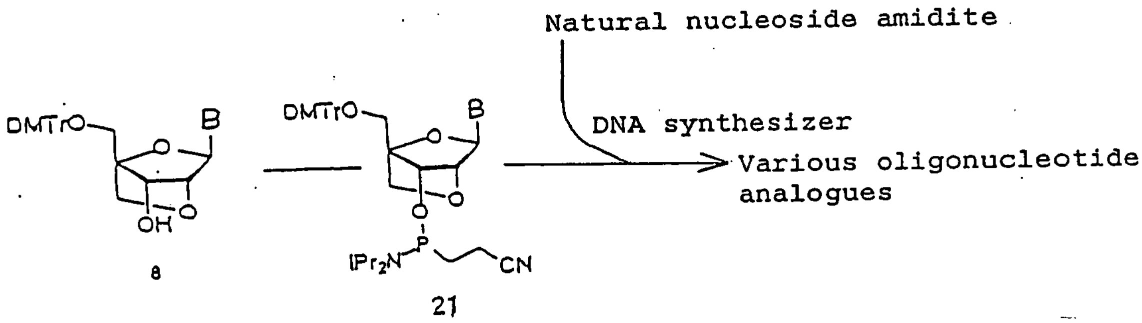
[0041]

IR ν (KBr):3048, 2935, 2852, 1749, 1466, 1369, 1234, 1108, 1040 cm^{-1}.

^{1}H-NMR ($CDCl_3$)δ: 1.06(9H,s), 1.94(3H,s), 2.98(1H,br s), 3.63, 4.00(2H,AB,J=10Hz), 3.72(1H,d,J=7Hz), 3.82-3.84(2H,m), 4.30 (1H,s), 5.25(1H,s), 7.40-7.46(6H,m), 7.60(4H,d,J=6Hz), 7.66 (1H,s), 9.68(1H,br s).

Example 3: Synthesis of oligonucleotide analogue

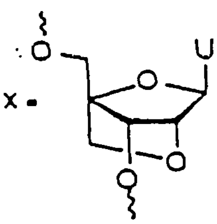
[Formula 11]



[Formula 12]

5'-GCGXTTTTTGCT-3' (XT5)
5'-GCGTTXTTTGCT-3' (T2XT3)
5'-GCGTTTXTTGCT-3' (T3XT2)
5'-GCGTTTTTXGCT-3' (T5X)
5'-GCGXXTTTTGCT-3' (X2T4)
5'-GCGTTXXTTGCT-3' (T2X2T2)
5'-GCGTTTTXXGCT-3' (T4X2)
5'-GCGXXXXXXGCT-3' (X6)
5'-GTTTTTTTTTXXC-3' (X2)

X =



(1) 3'-O-[2-cyanoethoxy(diisopropylamino)phosphino]-5'-O-(4,4'-dimethoxytrityl)-2'-O,4'-methanouridine (Compound 21)

Compound 8 (200 mg, 0.31 mmol) and diisopropylammonium tetrazolide (39.6 mg, 0.23 mmol) were subjected to azeotropy with anhydrous CH_3CN three times, and then the system was converted into an anhydrous CH_3CN-anhydrous THF solution (3:1, 4 ml). In a stream of nitrogen, 2-cyanoethyl N,N,N',N'-tetraisopropylphosphorodiamidite (0.12 ml, 0.37 mmol) was added, and the mixture was stirred for 90 minutes at room temperature. The solvents were distilled off under reduced pressure, and the resulting crude product was purified by silica gel column chromatography (AcOEt:hexane:Et_3N = 75:25:1). Then, the purified product was reprecipitated from AcOEt-hexane to

obtain an amidite compound 21 (181 mg, 0.25 mmol, 81%).

[0042]

m.p. 71-74°C (AcOEt-hexane).

^{31}P-NMR ($CDCl_3$): δ 149.6, 149.5, 149.4, 149.3, 149.2.

(2) General synthesis of oligonucleotide analogues

The synthesis of an oligomer was performed by means of Pharmacia's DNA synthesizer, Gene Assembler Plus, on a 0.2 μmol scale. The concentrations of solvents, reagents, and phosphoramidite were the same as for the synthesis of natural DNA. A DMTr group of 5'-O-DMTr-thymidine (0.2 μmol) having a 3'-hydroxyl group bound to a CPG support was deprotected with trichloroacetic acid. On its 5'-hydroxyl group, condensation reaction was repeated using an amidite comprising four nucleic acid bases for natural DNA synthesis and Compound 21 to synthesize oligonucleotide analogues of respective sequences. The synthetic cycle was as follows:

[0043]

<u>Synthetic cycle (0.2 μmol scale)</u>

1)	Detritylation	1% CCl_3COOH in CH_2ClCH_2Cl, 6 sec
2)	Coupling	0.1 M phosphoramidite (25 equiv.), 0.5 M 1H-tetrazole (500 equiv.) in MeCN, 2 min
3)	Capping	3% 4-(dimethylamino)pyridine, 10% Ac_2O, in MeCN, 18 sec
4)	Oxidation	0.01 M I_2 in 2,4,6-collidine/H_2O/MeCN (1:5:11), 6 sec

The synthesized oligomer was cleaved from the support by treatment with concentrated aqueous ammonia in the

customary manner. At the same time, the protective cyanoethyl group was detached from the phosphorus atom, and the protective groups for the adenine, guanine and cytosine were also removed.

[0044]

The resulting 5'-O-dimethoxytritylated oligonucleotide analogue was rid of the DMTr group by use of 5 ml trifluoroacetic acid on a reversed phase chromatographic column (Millipore, Oligo-Pak™SP), and further purified to obtain the desired oligonucleotide analogue.

[0045]

In accordance with the foregoing method for general synthesis, the following oligonucleotide analogues were synthesized:

[0046]

(2) 5'-GCGXTTTTTGCT-3' (XT5)
Yield 0.06 μmol (30% yield)

(3) 5'-GCGTTXTTTGCT-3' (T2XT3)
Yield 0.05 μmol (25% yield)

(4) 5'-GCGTTTXTTGCT-3' (T3XT2)
Yield 0.03 μmol (15% yield)

(5) 5'-GCGTTTTTXGCT-3' (T5X)
Yield 0.06 μmol (30% yield)

(6) 5'-GCGXXTTTTGCT-3' (X2T4)
Yield 0.06 μmol (30% yield)

(7) 5'-GCGTTXXTTGCT-3' (T2X2T2)
Yield 0.05 μmol (25% yield)

(8) 5'-GCGTTTTXXGCT-3' (T4X2)

Yield 0.06 μmol (30% yield)

(9) 5'-GCGXXXXXXGCT-3' (X6)

Yield 0.06 μmol (30% yield)

(10) 5'-GTTTTTTTTXXC-3' (X2)

Yield 0.07 μmol (35% yield)

Experimental Example 1: Measurement of melting temperature (Tm)

The melting temperatures (Tm's) of annealing products between antisense strands, which were the various oligonucleotide analogues synthesized in Example 2, and natural DNA- or RNA-based sense strands were measured to investigate the hybridizing ability of the oligonucleotide analogues of the present invention for complementary DNA and RNA.

[0047]

Each sample solution (500 μL) with end concentrations of 100 mM NaCl, 10 mM sodium phosphate buffer (pH 7.2), 4 μM antisense strand, and 4 μM sense strand, respectively, was bathed in boiling water, and slowly cooled to room temperature over the course of 10 hours. The sample solution was gradually cooled to 5°C, kept at 5°C for a further period of 20 minutes, and then started to be measured, with a stream of nitrogen being passed through a cell chamber of a spectrophotometer (UV-2100PC, Shimadzu) for prevention of moisture condensation. The sample temperature was raised at a rate of 0.2°C/minute until 90°C,

and the ultraviolet absorption at 260 nm was measured at intervals of 0.1°C. To prevent changes in the sample concentration with increases in the temperature, the cell was provided with a closure, and a drop of a mineral oil was applied onto the surface of the sample solution during measurement.

[0048]

The results are shown in the following table.

[0049]

Table 1 Melting Temperatures (Tm's) of Antisense Oligonucleotide Analogues for Complementary DNA and RNA

Antisense molecule	Tm for complementary DNA[a] (ΔTm/mod.)	Tm for complementary RNA[b] (ΔTm/mod.)
5'-GCGTTTTTTGCT-3' (natural)	47°C	45°C
5'-GCGXTTTTTGCT-3' (XT5)	50°C (+3°C)	49°C (+4°C)
5'-GCGTTXTTTGCT-3' (T2XT3)	49°C (+2°C)	49°C (+4°C)
5'-GCGTTTXTTGCT-3' (T3XT2)	49°C (+2°C)	50°C (+5°C)
5'-GCGTTTTTXGCT-3' (T5X)	52°C (+4°C)	51°C (+6°C)
5'-GCGXXTTTTGCT-3' (X2T4)	51°C (+2°C)	53°C (+4°C)
5'-GCGTTXXTTGCT-3' (T2X2T2)	49°C (+1°C)	53°C (+4°C)
5'-GCGTTTTXXGCT-3' (T4X2)	54°C (+3.5°C)	55°C (+5°C)
5'-GCGXXXXXXGCT-3' (X6)	58°C (+1.8°C)	71°C (+4.3°C)

a): 3'-CGCAAAAAACGA-5'. b):3'-r(CGCAAAAAACGA).

As shown in the table, in the case of the oligomer having one or two units (X) of the nucleoside analogue of the present invention (general formula (Ia)) introduced into a natural DNA strand, the ability to hybridize with the complementary DNA oligomer, evaluated by the Tm, rose by 2 to 7 degrees (about 2 degrees per modified residue) as compared with the natural strand. With the oligomer having all T's substituted by X's (X6), the increase in the ability was as high as 11 degrees. When the ability to hybridize with complementary RNA was evaluated, the oligomer incorporating one or two X's had an increase in Tm of 4 - 10 degrees (4 to 6 degrees per modified residue) over the natural strand. In the case of X6, the ability to hybridize with complementary RNA was further enhanced, showing an increase in Tm of more than 25 degrees (4 degrees per modified residue). There have been no examples of analogues undergoing such increases in Tm as compared with natural strands, and the affinity of the claimed oligomer was higher for RNA than for DNA. These facts mean that the oligonucleotide analogue composed of the bicyclooligonucleoside analogue of the present invention has extremely high performance as an antisense molecule, and is useful as a material for pharmaceuticals.

[0050]

Experimental Example 2: Measurement of nuclease resistance

A buffer solution (0.003 U/ml, 400 μl) of a snake venom phosphodiesterase was mixed with a buffer solution (10

μM, 400 μl) of the oligonucleotide held at 37°C for 15 minutes. The mixed solution was placed in a quartz cell (800 μl) kept at 37°C, and increases in the ultraviolet absorption (260 nm) due to the decomposition of the oligonucleotide were measured over time by means of SHIMADZU UV-2100PC. The buffer used comprised 0.1 M Tris-HCl (pH 8.6), 0.1 M NaCl, and 14 mM $MgCl_2$, and was sufficiently degassed before measurement.

[0051]

Measurement of half-life ($t_{1/2}$):

A calculation was made of the average of the values of the UV absorption measured at the start of measurement (t=0) and that measured at the time when no increase in this parameter was noted. The time corresponding to this average was designated as the half-life ($t_{1/2}$).

[0052]

Oligonucleotide sequence	$t_{1/2}$ (seconds)
5'-GTTTTTTTTTTC-3' (natural type)	260
5'-GTTTTTTTT-XX-C-3' (X2)	850

Charts showing the time course of the ultraviolet absorption are presented as Fig. 1 (natural strand) and Fig. 2 (X2). The ultraviolet absorption reached a plateau in about 30 minutes for the natural strand, and about 90 minutes for X2, after initiation of the enzyme reaction.

[Brief Description of the Drawings]

[Fig. 1]

A chart showing the time course of the ultraviolet

absorption (260 nm) of a naturally occurring oligonucleotide decomposed with an exonuclease.

[Fig. 2]

A chart showing the time course of the ultraviolet absorption (260 nm) of an oligonucleotide of the present invention (X2) decomposed with an exonuclease.

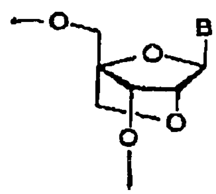
[Name of Document] Abstract

[Abstract]

[Object] An oligonucleotide analogue antisense molecule, which is minimally hydrolyzable with an enzyme in vivo, has a high sense strand binding ability, and is easily synthesized is provided.

[Construction] An oligo- or polynucleotide analogue having one or more structures of the general formula

[Formula 1]



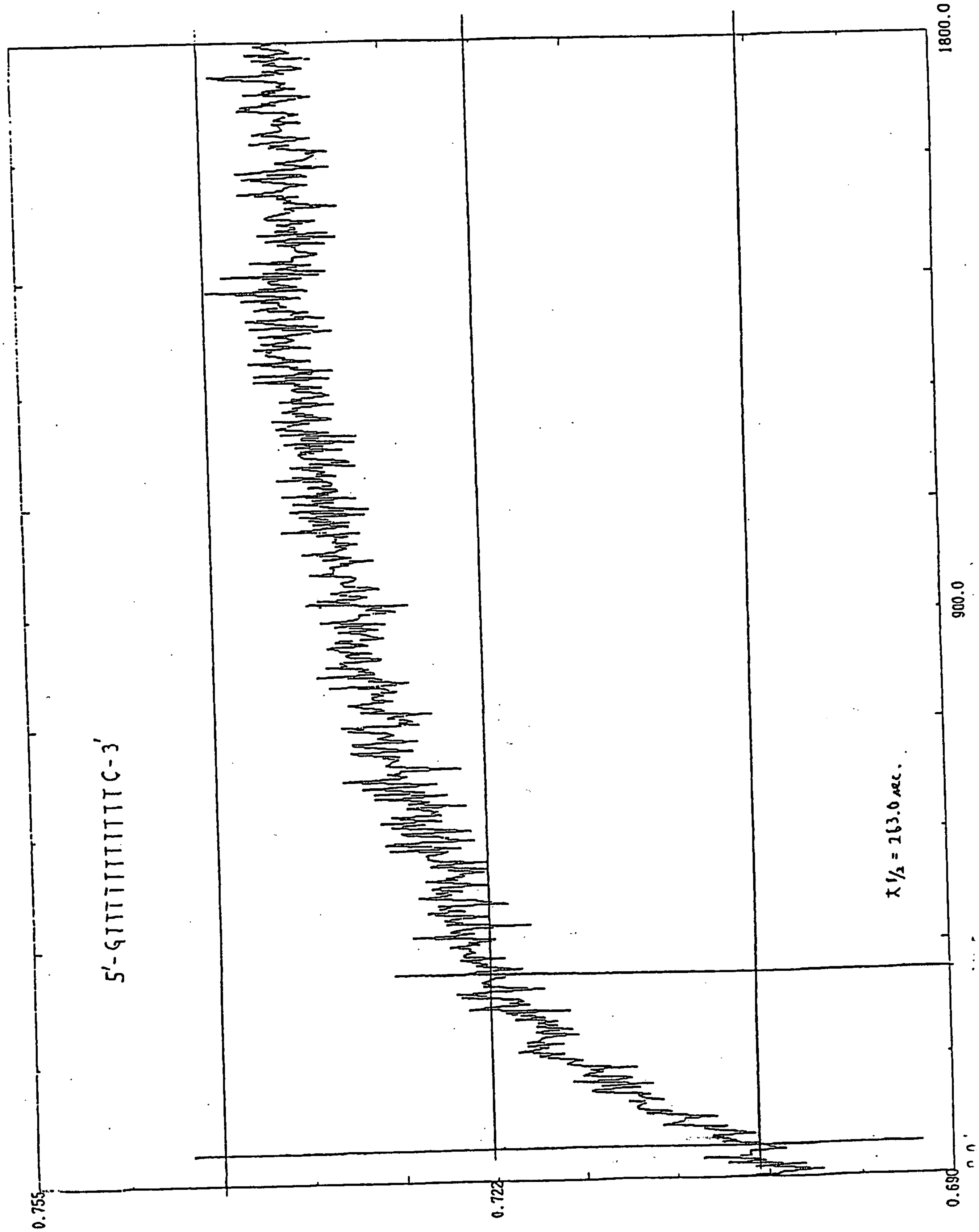
(Ia)

where B is a pyrimidine or purine nucleic acid base, or an analogue thereof.

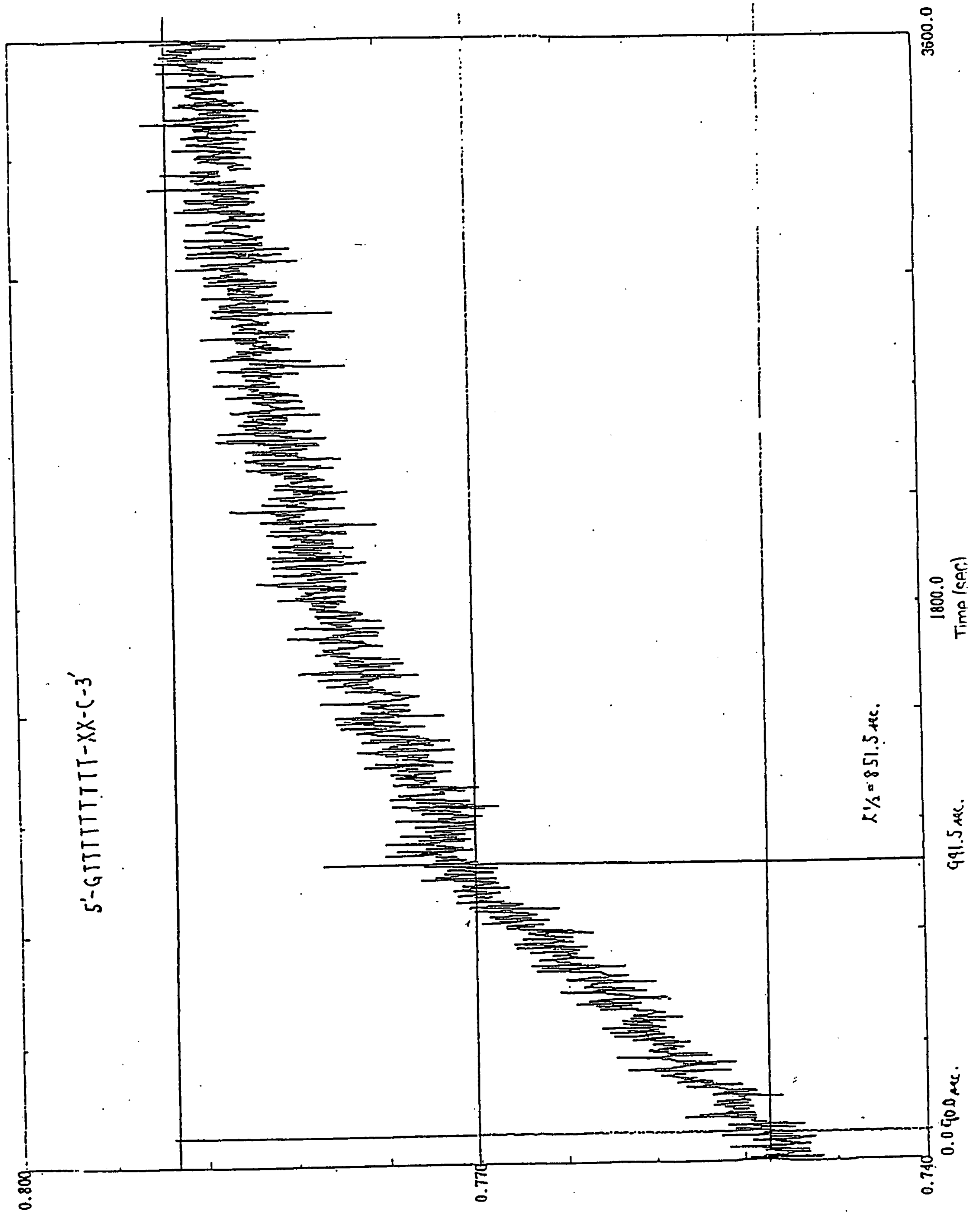
[Selected Drawing] None

[Name of Document] Drawing

[Fig. 1]



[Fig. 2]


5'-GTTTTTTTT-XX-C-3'
0.800
0.770
0.740
3600.0
1800.0
Time (sec)
0.0
90.0 sec.
991.5 sec.
t½ = 851.5 sec.